Standard Operating Procedure for Particulate-Phase Total Phosphorous by Persulfate Oxidation Digestion (Lachat Method)

LG209

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1.0 SCOPE AND APPLICATION

1.1 This method covers the determination of total phosphorous for particulate matrices in lake water.

1.2 The approximate working range is 1 to 200 µg/L.

2.0 SUMMARY

2.1 Samples are digested in the presence of sulfuric acid and ammonium persulfate to convert or "hydrolyze" polyphosphates and organic phosphorous to orthophosphate.

2.2 The orthophosphate ion $(PO_4)^{3-}$ reacts with ammonium molybdate and antimony potassium tartrate under acidic conditions to form 12-molybdophosphoric acid. This complex is reduced with ascorbic acid to form a blue complex which absorbs light at 880 nm. The absorbance is proportional to the concentration of orthophosphate in the sample.

3.0 SAMPLE HANDLING AND COLLECTION

3.1 Samples are filtered by vacuum through 47-mm cellulose acetate filters with a 0.45-µm pore size.

3.2 The filters retaining the particles are collected into clean Petri dishes, and frozen until analysis.

4.0 INTERFERENCES

4.1 Silica forms a pale blue complex which also absorbs at 880 nm. This interference is generally insignificant. A silica concentration of 50 mg SiO_2/L is required to produce a 0.008 mg P/L positive error in orthophosphorous.

4.2 To minimize contamination and interference commonly associated with the use of commercial detergents, all glassware should be washed with 1:1 HCl, rinsed with reagent water and dried. **Treated glassware (e.g., volumetric flasks, graduated cylinders) designated for use in low level TP analysis (labeled 'TP ONLY') can be found segregated in the prep area.**

4.3 High concentrations of the ferric ion or arsenate ion can cause error due to competition between these ions and ascorbic acid for the complex. Such concentrations are highly unlikely in lake water.

5.0 APPARATUS

5.1 60-mL test tubes with polypropylene Teflon-lined screw caps

5.2 Cellulose acetate filters with 0.45-µm pore size

5.3 Autoclave

5.4 Calibrated automatic pipets with disposable plastic tips

5.5 Lachat QuikChem FIA+ (8000 series)

5.5.1 Phosphate Manifold (Lachat Manifold # 30-115-01-1-B)

5.5.2 Printer

5.5.3 XYZ Sampler

6.0 REAGENTS AND STANDARDS

6.1 All reagents should be stored in the appropriate bottles and labeled with the following information:

Identity:	(Sulfuric Acid)
Concentration:	(4.4 N)
Date of Preparation:	(mm/dd/yy)
Expiration Date:	(mm/dd/yy)
Initials of Preparer:	(IMF)

6.2 **Use reagent water for all solutions.**

6.3 Sulfuric Acid Solution. With continuous mixing, add 150 ml of concentrated sulfuric acid to 385 ml of Reagent water. **Very high temperatures are generated when sulfuric acid is mixed with water. Never add water to containers of sulfuric acid. This operation should be performed in a fume hood.**

6.4 **Ammonium Persulfate Concentrate:** In a 100 ml volumetric flask, dissolve 20.0 g ammonium persulfate [$(NH_4)_2S_2O_8$] in about 80 ml of regent water. Dilute to the mark.

6.5 **Digestion Solution:** Combine one part of Sulfuric Acid Solution (6.3) and one part of Ammonium Persulfate Concentrate (6.4).

6.6 **Stock Ammonium Molybdate Solution:** In a 1-L volumetric flask dissolve 40.0 g ammonium molybdate tetrahydrate [$(NH_4)Mo_7O_{24}$] in approximately 800 mL of reagent water. Dilute to the mark and invert to mix. Store in plastic bottle at 4°C.

6.7 **Stock Antimony Potassium Tartrate Solution:** In a 1-L volumetric flask, containing approximately 800 mL of water dissolve 3.0 g antimony potassium tartrate [$K(SbO)C_4H_4O_6 \cdot ½H_2O$]. Dilute to the mark. Store in a dark bottle at 4°C.

6.8 **Molybdate Color Reagent:** In a 500 mL volumetric flask containing about 100 ml of reagent water, add 75.0 ml of Sulfuric Acid Solution (6.3). Swirl to mix. Add 36.0 ml of the **Stock Antimony Potassium Tartrate Solution** and 100.0 ml of the **Stock Ammonium Molybdate Solution.** Dilute to the mark. Degas with helium.

6.9 **Ascorbic Acid Reducing Solution:** In a 1-L volumetric flask dissolve 60.0 g ascorbic acid in about 700 mL of reagent water. Dilute to the mark and invert to mix. Degas with helium. Add 1.0 g dodecyl sulfate, sodium salt ($CH_3(CH_2)_{11}OSO_3Na$).

6.10 **Sulfuric Acid Carrier Solution:** In a 1 L volumetric flask containing about 950 ml of reagent water, add 50 ml of autoclaved Digestion Solution (6.5). Dilute to the mark and degas with helium.

6.11 **Sodium Hydroxide - EDTA Rinse:** Dissolve 65 g sodium hydroxide (NaOH) and 6 g tetrasodium ethylenediamine tetra-acetic acid (Na_4EDTA) in 1 L of reagent water.

6.12 Preparation of Standards

6.12.1 **Stock 1000 mg P/L Calibration Standard:** Dry a small amount of potassium dihydrogen phosphate (KH_2PO_4) in an oven at 105°C to constant weight. In a 1-L volumetric flask, dissolve 4.394 g of dried reagent in approximately 500 mL of reagent water. Add 1.0 mL of concentrated sulfuric acid and dilute to the mark. Store at 4°C.

6.12.2 **Intermediate 10.0 mg P/L Calibration Standard:** Into a 1-L volumetric flask pipet 10 mL of the Stock Calibration Standard (6.11.1) and dilute to the mark. Store at 4°C.
Alternatively Intermediate Calibration Standard (10.0 mg P/L) can be prepared from commercial 50 mg P/L Phosphate Standard. Transfer 40 ml of **Commercial 50 mg P/L Phosphate Standard** into a 200 mL volumetric flask, containing about 100 mL of regent water and dilute to the mark. Store at 4°C.

6.12.3 **Working Calibration Standards:** Prepare standards over the range of analysis. For the working range of 0 - 200 µg/L, the following standards may be used:

mL of Intermediate Calibration Standard (6.11.2) diluted to 1 L	Concentration µg P/L
0.0	0.0
1.0	10.0
2.5	25.0
5.0	50.0
10.0	100.0
15.0	150.0
20.0	200.0

6.12.4 **Stock 100 mg P/L Control Standard:** In a 1-L volumetric flask, dissolve 1.3793 g of Adenosine-5-Monophosphoric Acid, Disodium salt, [$C_{10}H_{12}N_5O_7PNa_2 \bullet 2H_2O$,F.W. 427.236 g/mole, Fluka] in about 500 mL of reagent water. Store at 4°C.
Alternatively Stock 100 mg P/L Control Standard can be prepared from **Commercial 1000 mg P/L Organic Nutrients Standard.** Transfer 100 ml of commercial 1000 mg P/L Organic Nutrients Standard into a 1 L volumetric flask, containing about 500 ml of regent water and dilute to the mark. Store at 4°C.

6.12.5 **Intermediate 10.0 mg P/L Control Standard:** Transfer 100 mL of the Stock Control Standard (6.12.4) into a 1-L volumetric flask and dilute to the mark. Store at 4°C. The **Spike** is prepared by adding 0.20 mL of this standard to 38.0 mL of selected routine field samples.

6.12.6 Working Control Standards: The following concentrations are typical:

QC Type	mL of Intermediate Control Standard (6.11.5) Diluted to 1 L	Concentration in μg P/L
High Check Standard (CH)	12.0	120.0
Low Check Standard (CL)	4.0	40.0

7.0 PROCEDURE

7.1 The filters should be carefully transferred to the cleans and dried digestion tubes. To each tube add 38 mL of reagent water and 2.0 mL of digestion (6.5). Recap the tube.

7.2 Insert one plain cellulose acetate filter to each cleansed and dried tubes. Add 38 mL of working calibration standards, control standards and reagent blanks. Add 4.0 mL of digestion solution to each tube (6.5) and close with cap.

7.3 Autoclave samples and standards for 30 minutes at 15 psi and 121°C. Bring samples and standards to the room temperature.

7.4 Follow the Lachat Procedural SOP.

8.0 CALCULATIONS

8.1 The computer yields results directly in µg P/L.

8.2 Calculate the Particulate total phosphorous concentration using the following equation:

$$C_{PTP} = \frac{C_P}{25{,}000 \times V}$$

Where:

C_{PTP} - Concentration of particulate total phosphorous in water (mg P/L)
C_P - Concentration of phosphorous from LACHAT instrument (□g P/L)
V - Volume of filtered water (L)

9.0 QUALITY CONTROL

9.1 Refer to the Chapter 2 Introduction for definitions of quality control samples and information regarding quality control procedures, such as QC sample IDs and labeling**.**

9.2 The following QC samples must be prepared and analyzed at the minimum frequency indicated.

QC Sample Type		Frequency	Acceptance Criteria
External	**Field Reagent Blank (FRB)**	**One per basin[a]**	**0.0 ± 2.0 □g/L or less than one tenth associated field sample concentrations, whichever is greater**
	Lab Duplicate (LD1)	**One per basin[a]**	**RPD□ 20%**
Internal	**Calibration**	**At the beginning of each batch**	**r□ 0.995**
	High Check Standard (CH)	**At the beginning & end of each batch or 1 per 40 samples, whichever is more frequent**	**120.0 ± 12.0 □g/L**
	Low Check Standard (CL)	**At the beginning & end of each batch or 1 per 40 samples, whichever is more frequent**	**40.0 ± 4.0 □g/L**
	Laboratory Reagent Blank (LRB)	**At the beginning & end of each batch or 1 per 40 samples, whichever is more frequent**	**0.00 ± 2.0 □g/L**
	Matrix Spike (MS)	**During each batch or 1 per 40 samples, whichever is more frequent**	**100% ± 20%**
	Method Detection Limit (MDL)	**Once per year and each time a significant change is made to the SOP**	**1 □g/L**

[a] A field duplicate, lab duplicate, and field reagent blank are collected with each group of 3, 4, or 5 stations depending on the lake. A Random Number Generator (RNG) is used to determine the stations and depths of these QC samples. Where basins are well defined, at least one of each is collected from each basin.

9.3 Assessment

9.3.1 The analyst must compare analytical results to the acceptance criteria listed in Section 9.2 to identify QC failures. If the results are outside the acceptance criteria, the analyst should first review their calculations for errors and if none are identified, they must follow the corrective action procedures listed in Section 9.4.

9.4 Corrective Actions

9.4.1 Corrective action procedures will often be handled at the bench level by the analyst, who reviews the preparation or extraction procedure for possible errors, checks the instrument calibration, spike and calibration mixes, instrument sensitivity, and any other potential sources of error. If failure occurs and an error is identified, the analyst should re-run quality control and RFS samples in the entire analytical batch to confirm the results. Because external QC samples are collected and prepared during the survey and provided to the contractor or grantee laboratory, a single rerun to confirm

results is sufficient when all other QC samples are within acceptance criteria. For analyses conducted onboard, if the problem persists or cannot be identified, the matter must be referred to the Chief Scientist for further investigation. Depending upon the Chief Scientist's evaluation, the analyst may or may not be required to prepare and re-run the samples again. Additionally, if the results are significantly different than the expected concentrations based on historical data or related samples, then the analyst may split the RFS sample in the laboratory and analyze the splits. Once a decision is made, full documentation of the corrective action procedures and assessment of the final result must be filed with the WQS QM Technical Lead (Marvin Palmer) or the GLNPO QM. For analyses conducted at contract or grantee laboratories, this information can be included with submitted data. When contractor or grantee laboratories have a question regarding acceptable corrective actions, they should contact the Biology Technical Lead or Limnology Technical Lead as appropriate for instruction at a time when corrective action can still be taken.

9.5 Data Reporting/Recording

9.5.1 When corrective actions are not feasible or do not resolve QC failure, the analyst is responsible for identifying all failed QC samples and RFS samples. If analyses are being conducted onboard, the analyst should document the QC information on the hard-copy Field Information Recording Forms (Appendix H). If analyses are being conducted by contract or grantee laboratories, the analyst should document all QC information with the submitted data.

10.0 WASTE DISPOSAL

10.1 Effluent waste and sampler waste from this analysis are acidic. Dispose of these waste products by pouring them directly into the acid waste carboys bearing yellow labels. To prevent spills and injuries, use personal safety precautions and wear all prescribed personal safety gear in handling acidic wastes.

11.0 PREVENTATIVE MAINTENANCE

11.1 Required maintenance is described in the Lachat Procedural SOP.

12.0 TROUBLESHOOTING

12.1 The heating coil may need to be freshly retubed. When cleaning the system in the prescribed manner fails to optimize the baseline, the pump and the manifold.

12.2 Negative sample peaks are observed when matrix differences between the carrier solution and the digested samples exist. Be sure the carrier solution has been properly prepared, using high purity reagents, reagent water, and appropriately treated glassware.

12.3 An unusually noisy baseline may be due to insufficient helium sparging of on-line reagents. Be sure all reagents to be pumped through manifold tubing have been sufficiently degassed with helium.

13.0 REFERENCES

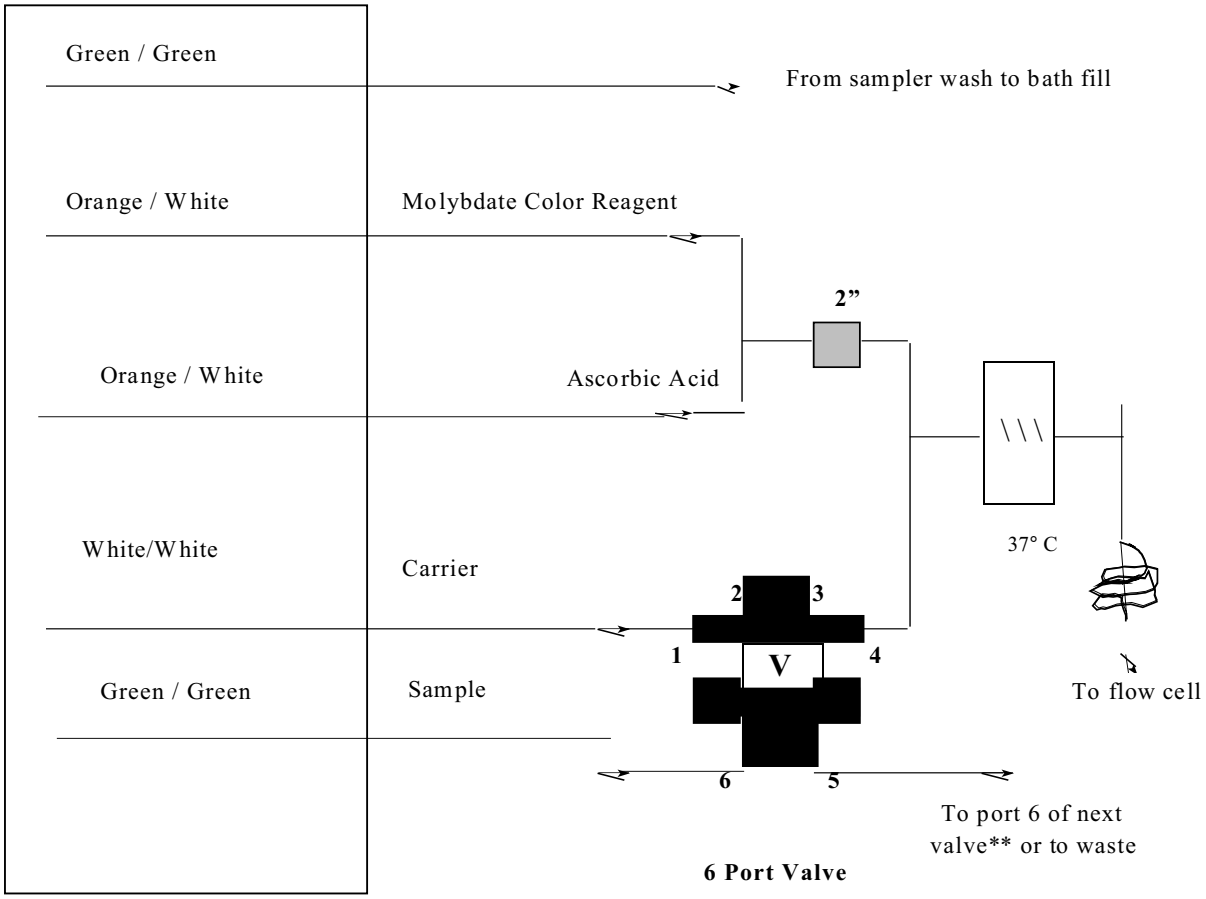
13.1 Lachat Instruments, Method Number 10-115-01-1-F, Total Phosphorus in Persulfate Digest, Revision Date May 1992.

13.2 Lachat QuikChem AE Operating Manual.

13.3 GLAS Standard Operating Procedure, Total Phosphorus, Low-Level Micro-persulfate digestion. August 1990.

13.4 Standard Operating Procedure for Determining Total Phosphorous, Available Phosphorous, and Biogenic Silica Concentration of Lake Michigan Sediments and Sediment Trap Material. Method Code: SEDNUT96. January, 1996.

14.0 TOTAL PHOSPHORUS ANALYTICAL MANIFOLD



Legend

- 2.0" Mixing coil (there is 135 cm of tubing on the 2.0" coil support)

- The box shows 175 cm of tubing warped around the block heater.

Comments

1. Filter used is 880 nm.
2. Sample loop length is 300 cm.
3. All manifold tubing is 0.52 mm (0.022") ID.
4. 3 m back pressure loop 0.52mm (0.22") ID.
5. The carrier is Reagent 6.10.

** If more than one channel is being used.